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## THE INFLUENCE OF INCUBATION ON THE WASSERMANN REACTION

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Smith and MacNeal<sup>1</sup> demonstrated that widely different results may be obtained in complement-fixation for syphilis by the use of different incubation methods. First incubation conducted in the refrigerator at a low temperature gave many more positive results than did first incubation conducted at a temperature of 37 C. In a later communication<sup>2</sup> the same authors showed that cholesterinized antigen, plain alcoholic extract, and the acetone insoluble antigen bind much more complement at low temperature than at a temperature of 37 C. In the refrigerator, plain alcoholic extract bound more complement than did cholesterinized antigen in the incubator.

In this paper I shall present the results I have obtained with the Wassermann reaction under different incubation conditions. Only one antigen was employed, and the only difference there was, was in incubation time and method.

The results are presented under 5 different heads. Under the first head are given results obtained by comparing incubation in the incubator at a temperature of 37 C. with incubation in the open water-bath at a temperature of 37 C. The second deals with results obtained in the incubator at 37 C. and in the refrigerator at 10 C. Here each serum was tested 10 times, first incubation in incubator 0.25 hour, 0.5 hour, 1 hour, 2 hours and 4 hours, and these were duplicated in the refrigerator. In the third group each serum was tested 4 times; first incubation was carried on in the incubator for 1 and 4 hours, and in the refrigerator for 1 and 4 hours. In the fourth group the incubation time only was varied. First incubation was carried on in the refrigerator for 5 and 10 hours. In the fifth group first incubation at a temperature of 21 C. for 5 hours was compared with first incubation at a temperature of 10 C. for 5 hours. All human serums used in these tests were glycerolated, and in Tests 1 and 2 each tube received 0.1 c.c. of serum-glycerol mixture while in Tests 3, 4, and 5 each tube received 0.25 c.c. of serum-glycerol mixture and the total quantity in each tube was brought up to 1.25 c.c.

### TEST 1

Serums 1 to 20, inclusive, were tested 4 times. First incubation was in the incubator at 37 C. for 1 and 2 hours, and in the water-bath at 37 C. for 1 and 2 hours. After having added the sensitized blood corpuscles the

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<sup>1</sup> Jour. Immunol., 1916, 2, p. 75.

<sup>2</sup> Jour. Infect. Dis., 1917, 21, p. 233.

TABLE 1  
INCUBATION IN INCUBATOR COMPARED WITH INCUBATION IN WATER-BATH

Number	Incubation Method I = Incubator W = Water-bath	Incubation Time, Hours	Amboceptor per Tube Unit	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
1	I	1	1.25	+	+	tr	+	+	tr	Negative,	—
	W	1	1.25	+	+	0	+	+	0	Negative,	—
	I	2	1.25	+	+	tr	+	+	tr	Negative,	—
	W	2	1.25	+	+	0	+	+	0	Negative,	—
2	I	1	1.25	+	+	tr	+	+	tr	Negative,	—
	W	1	1.25	+	+	0	+	+	0	Negative,	—
	I	2	1.25	+	+	tr	+	+	tr	Negative,	—
	W	2	1.25	+	+	0	+	+	0	Negative,	—
3	I	1	1.25	+	+	tr	+	+	tr	Negative,	—
	W	1	1.25	+	+	0	+	+	0	Negative,	—
	I	2	1.25	+	+	tr	+	+	tr	Negative,	—
	W	2	1.25	+	+	0	+	+	0	Negative,	—
4	I	1	1.25	+	+	±	+	+	±	Negative,	—
	W	1	1.25	+	+	tr	+	+	tr	Negative,	—
	I	2	1.25	+	+	±	+	+	±	Negative,	—
	W	2	1.25	+	+	tr	+	+	tr	Negative,	—
5	I	1	1.25	+	+	±	+	+	±	Negative,	—
	W	1	1.25	+	+	tr	+	+	tr	Negative,	—
	I	2	1.25	+	+	±	+	+	±	Negative,	—
	W	2	1.25	+	+	tr	+	+	tr	Negative,	—
6	I	1	1.25	+	+	±	+	+	±	Negative,	—
	W	1	1.25	+	+	tr	+	+	tr	Negative,	—
	I	2	1.25	+	+	±	+	+	±	Negative,	—
	W	2	1.25	+	+	tr	+	+	tr	Negative,	—
7	I	1	1.25	+	+	±	+	+	±	Negative,	—
	W	1	1.25	+	+	tr	+	+	tr	Negative,	—
	I	2	1.25	+	+	±	+	+	±	Negative,	—
	W	2	1.25	+	+	tr	+	+	tr	Negative,	—
8	I	1	1.25	+	+	±	+	+	±	Negative,	—
	W	1	1.25	+	+	tr	+	+	tr	Negative,	—
	I	2	1.25	+	+	±	+	+	±	Negative,	—
	W	2	1.25	+	+	tr	+	+	tr	Negative,	—
9	I	1	1.25	+	+	tr	+	+	tr	Negative,	—
	W	1	1.25	+	+	0	+	+	0	Negative,	—
	I	2	1.25	+	+	tr	+	+	tr	Negative,	—
	W	2	1.25	+	+	0	+	+	0	Negative,	—
10	I	1	1.25	+	+	tr	+	+	tr	Negative,	—
	W	1	1.25	+	+	0	+	+	0	Negative,	—
	I	2	1.25	+	+	tr	+	+	tr	Negative,	—
	W	2	1.25	+	+	0	+	+	0	Negative,	—
11	I	1	1.25	+	+	tr	+	+	±	Weakly positive,	+
	W	1	1.25	+	+	tr	+	+	±	Weakly positive,	+
	I	2	1.25	+	+	tr	+	+	±	Weakly positive,	+
	W	2	1.25	+	+	0	+	+	tr	Weakly positive,	+
12	I	1	1.25	+	±	0	+	+	±	Strongly positive,	+++
	W	1	1.25	+	+	0	+	+	±	Moderately positive,	++
	I	2	1.25	+	tr	0	+	+	±	Strongly positive,	+++
	W	2	1.25	+	+	0	+	+	±	Moderately positive,	++
13	I	1	1.25	+	+	0	+	+	±	Moderately positive,	++
	W	1	1.25	+	+	0	+	+	±	Moderately positive,	++
	I	2	1.25	+	+	0	+	+	±	Moderately positive,	++
	W	2	1.25	+	+	0	+	+	±	Moderately positive,	++

Explanation: + means complete hemolysis; ±, hemolysis between 50% and 100%; tr (trace), hemolysis 50% or less; 0, no hemolysis.

TABLE 1—Continued  
INCUBATION IN INCUBATOR COMPARED WITH INCUBATION IN WATER-BATH

Number	Incubation Method I = Incubator W = Water-bath	Incubation Time, Hours	Amboceptor per Tube Unit	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
14	I	1	1.25	+	+	0	+	+	tr	Weakly positive,	+
	W	1	1.25	+	+	0	+	+	tr	Weakly positive,	+
	I	2	1.25	+	+	0	+	+	±	Moderately positive,	++
	W	2	1.25	+	+	0	+	+	tr	Weakly positive,	+
15	I	1	1.25	+	±	0	+	+	tr	Moderately positive,	++
	W	1	1.25	+	±	0	+	+	tr	Moderately positive,	++
	I	2	1.25	+	+	0	+	+	±	Moderately positive,	++
	W	2	1.25	+	±	0	+	+	tr	Moderately positive,	++
16	I	1	1.25	+	0	0	+	+	tr	Strongly positive,	++++
	W	1	1.25	+	tr	0	+	+	tr	Strongly positive,	++++
	I	2	1.25	+	0	0	+	+	±	Strongly positive,	+++++
	W	2	1.25	+	tr	0	+	+	tr	Strongly positive,	++++
17	I	1	1.25	+	+	0	+	+	±	Moderately positive,	++
	W	1	1.25	+	±	0	+	+	tr	Moderately positive,	++
	I	2	1.25	+	±	0	+	+	±	Strongly positive,	+++
	W	2	1.25	+	±	0	+	+	tr	Moderately positive,	++
18	I	1	1.25	+	+	0	+	+	±	Moderately positive,	++
	W	1	1.25	+	+	0	+	+	tr	Weakly positive,	+
	I	2	1.25	+	+	0	+	+	±	Moderately positive,	++
	W	2	1.25	+	+	0	+	+	tr	Weakly positive,	+
19	I	1	1.25	+	+	tr	+	+	±	Weakly positive,	+
	W	1	1.25	+	+	0	+	+	tr	Weakly positive,	+
	I	2	1.25	+	+	0	+	+	tr	Moderately positive,	++
	W	2	1.25	+	+	0	+	+	tr	Weakly positive,	+
20	I	1	1.25	+	±	0	+	+	tr	Moderately positive,	++
	W	1	1.25	+	tr	0	+	+	0	Moderately positive,	++
	I	2	1.25	+	±	0	+	+	tr	Moderately positive,	++
	W	2	1.25	+	tr	0	+	+	0	Moderately positive,	++

Explanation: + means complete hemolysis; ±, hemolysis between 50% and 100%; tr (trace), hemolysis 50% or less; 0, no hemolysis.

tubes from the incubator were returned to the incubator, and the tubes from the water-bath were returned to the water-bath.

Table 1 shows the results obtained in the incubator and in the water-bath with Serums 1-20, inclusive. The first 10 serums were from nonsyphilitic persons, and they all gave negative results. Serums 11 to 20, inclusive, were from persons known to be syphilitic. During the first incubation, complement was bound better in the incubator than in the water bath; Serum 12 gave +++ and ++++ in the incubator, and ++ and ++ in the water-bath; Serums 14, 16, 17, 18, and 19 also gave stronger positive results in the incubator than in the water-bath. In no instance was the result obtained in the water-bath stronger positive than that obtained in the incubator.

## TEST 2

In order to observe the rate at which complement is bound Serums 21-30, inclusive, were incubated in the incubator at a temperature of 37 C. and in the refrigerator at a temperature of 10 C. for 0.25 hour, 0.5 hour, 1 hour, 2 hours, and 4 hours. After the addition of sensitized blood corpuscles all tubes were placed in the incubator because hemolysis was better in the incubator than in the water-bath. All of these serums were obtained from syphilitic patients under treatment.

TABLE 2  
INCUBATION IN INCUBATOR COMPARED WITH INCUBATION IN REFRIGERATOR

Number	Incubation Method I = Incubator R = Refrigerator	Incubation Time, Hours	Amboceptor per Tube Unit	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
21	I	0.25	1.25	+	+	±	+	+	±	Negative,	—
	R	0.25	1.25	+	+	±	+	+	±	Negative,	—
	I	0.5	1.25	+	+	±	+	+	±	Negative,	—
	R	0.5	1.25	+	+	±	+	+	±	Negative,	—
	I	1	1.25	+	+	±?	+	+	±	Faintly positive,	±
	R	1	1.25	+	+	±?	+	+	±	Faintly positive,	±
	I	2	1.25	+	+	tr	+	+	±	Weakly positive,	+
	R	2	1.25	+	+	tr	+	+	±	Weakly positive,	+
	I	4	1.25	+	±	0	+	+	tr	Moderately positive,	++
	R	4	1.25	+	+	0	+	+	±	Moderately positive,	++
22	I	0.25	1.25	+	+	±	+	+	±	Negative,	—
	R	0.25	1.25	+	+	±	+	+	±	Negative,	—
	I	0.5	1.25	+	+	±?	+	+	±	Faintly positive,	±
	R	0.5	1.25	+	+	±?	+	+	±	Faintly positive,	±
	I	1	1.25	+	+	tr	+	+	±	Weakly positive,	+
	R	1	1.25	+	+	tr	+	+	±	Weakly positive,	+
	I	2	1.25	+	+	tr	+	+	±	Weakly positive,	+
	R	2	1.25	+	+	tr	+	+	±	Weakly positive,	+
	I	4	1.25	+	±	0	+	+	tr	Moderately positive,	++
	R	4	1.25	+	+	0	+	+	±	Moderately positive,	++
23	I	0.25	1.25	+	+	±	+	+	±	Negative,	—
	R	0.25	1.25	+	+	±	+	+	±	Negative,	—
	I	0.5	1.25	+	+	±	+	+	±	Negative,	—
	R	0.5	1.25	+	+	±	+	+	±	Negative,	—
	I	1	1.25	+	+	±?	+	+	±	Faintly positive,	±
	R	1	1.25	+	+	±?	+	+	±	Faintly positive,	±
	I	2	1.25	+	+	0	+	+	tr	Weakly positive,	+
	R	2	1.25	+	+	tr	+	+	±	Weakly positive,	+
	I	4	1.25	+	+	0	+	+	tr	Weakly positive,	+
	R	4	1.25	+	+	tr	+	+	±	Weakly positive,	+
24	I	0.25	1.25	+	+	±	+	+	±	Negative,	—
	R	0.25	1.25	+	+	±	+	+	±	Negative,	—
	I	0.5	1.25	+	+	±	+	+	±	Negative,	—
	R	0.5	1.25	+	+	±	+	+	±	Negative,	—
	I	1	1.25	+	+	tr?	+	+	tr	Faintly positive,	±
	R	1	1.25	+	+	±?	+	+	±	Faintly positive,	±
	I	2	1.25	+	±	0	+	+	0	Weakly positive,	+
	R	2	1.25	+	+	0	+	+	tr	Weakly positive,	+
	I	4	1.25	+	tr	0	+	+	0	Moderately positive,	++
	R	4	1.25	+	±	0	+	+	tr	Moderately positive,	++
25	I	0.25	1.25	+	+	tr	+	+	±	Weakly positive,	+
	R	0.25	1.25	+	+	tr	+	+	±	Weakly positive,	+
	I	0.5	1.25	+	+	0	+	+	±	Moderately positive,	++
	R	0.5	1.25	+	±	0	+	+	±	Strongly positive,	++++
	I	1	1.25	+	±	0	+	+	±	Strongly positive,	++++
	R	1	1.25	+	0	0	+	+	±	Strongly positive,	+++++
	I	2	1.25	+	tr	0	+	+	±	Strongly positive,	+++++
	R	2	1.25	+	0	0	+	+	±	Strongly positive,	+++++
	I	4	1.25	+	0	0	+	+	tr	Strongly positive,	+++++
	R	4	1.25	±	0	0	+	+	±	Strongly positive,	+++++
26	I	0.25	1.25	+	±	0	+	+	tr	Moderately positive,	++
	R	0.25	1.25	+	±	0	+	+	tr	Moderately positive,	++
	I	0.5	1.25	+	±	0	+	+	tr	Moderately positive,	++
	R	0.5	1.25	+	±	0	+	+	tr	Moderately positive,	++
	I	1	1.25	+	±	0	+	+	tr	Moderately positive,	++
	R	1	1.25	+	±	0	+	+	tr	Moderately positive,	++
	I	2	1.25	+	tr	0	+	+	0	Moderately positive,	++
	R	2	1.25	+	±	0	+	+	tr	Moderately positive,	++
	I	4	1.25	+	0	0	+	+	0	Strongly positive,	++++
	R	4	1.25	+	tr	0	+	+	tr	Strongly positive,	++++

Explanation: + means complete hemolysis; ±, hemolysis between 50% and 100%; tr (trace), hemolysis 50% or less; 0, no hemolysis.

TABLE 2—Continued

INCUBATION IN INCUBATOR COMPARED WITH INCUBATION IN REFRIGERATOR

Number	Incubation Method I = Incubator R = Refrigerator	Incubation Time, Hours	Amboceptor per Tube Unit	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
27	I	0.25	1.25	+	+	0	+	+	tr	Weakly positive,	+
	R	0.25	1.25	+	+	0	+	+	tr	Weakly positive,	+
	I	0.5	1.25	+	±	0	+	+	tr	Moderately positive,	++
	R	0.5	1.25	+	±	0	+	+	tr	Moderately positive,	++
	I	1	1.25	+	±	0	+	+	tr	Moderately positive,	++
	R	1	1.25	+	±	0	+	+	tr	Moderately positive,	++
	I	2	1.25	+	tr	0	+	+	0	Moderately positive,	++
	R	2	1.25	+	tr	0	+	+	tr	Strongly positive,	+++
	I	4	1.25	+	0	0	+	+	0	Strongly positive,	+++
R	4	1.25	+	tr	0	+	+	tr	Strongly positive,	+++	
28	I	0.25	1.25	+	+	±?	+	+	±	Faintly positive,	±
	R	0.25	1.25	+	+	±?	+	+	±	Faintly positive,	±
	I	0.5	1.25	+	+	tr	+	+	±	Weakly positive,	+
	R	0.5	1.25	+	±	0	+	+	±	Strongly positive,	+++
	I	1	1.25	+	±	0	+	+	±	Strongly positive,	+++
	R	1	1.25	+	tr	0	+	+	±	Strongly positive,	+++
	I	2	1.25	+	±	0	+	+	±	Strongly positive,	+++
	R	2	1.25	+	tr	0	+	+	±	Strongly positive,	+++
	I	4	1.25	+	tr	0	+	+	tr	Strongly positive,	+++
R	4	1.25	+	0	0	+	+	±	Strongly positive,	+++++	
29	I	0.25	1.25	+	+	±?	+	+	±	Faintly positive,	±
	R	0.25	1.25	+	+	±?	+	+	±	Faintly positive,	±
	I	0.5	1.25	+	+	±?	+	+	±	Faintly positive,	±
	R	0.5	1.25	+	+	±?	+	+	±	Faintly positive,	±
	I	1	1.25	+	+	tr	+	+	±	Weakly positive,	+
	R	1	1.25	+	+	tr	+	+	±	Weakly positive,	+
	I	2	1.25	+	+	tr	+	+	±	Weakly positive,	+
	R	2	1.25	+	+	tr	+	+	±	Weakly positive,	+
	I	4	1.25	+	+	0	+	+	±	Moderately positive,	++
R	4	1.25	+	+	0	+	+	±	Moderately positive,	++	
30	I	0.25	1.25	+	+	±?	+	+	±	Faintly positive,	±
	R	0.25	1.25	+	+	±?	+	+	±	Faintly positive,	±
	I	0.5	1.25	+	+	±?	+	+	±	Faintly positive,	±
	R	0.5	1.25	+	+	±?	+	+	±	Faintly positive,	±
	I	1	1.25	+	+	tr	+	+	±	Weakly positive,	+
	R	1	1.25	+	+	tr	+	+	±	Weakly positive,	+
	I	2	1.25	+	+	tr	+	+	±	Weakly positive,	+
	R	2	1.25	+	+	0	+	+	±	Moderately positive,	++
	I	4	1.25	+	±	0	+	+	tr	Moderately positive,	++
R	4	1.25	+	+	0	+	+	±	Moderately positive,	++	

Explanation: + means complete hemolysis; ±, hemolysis between 50% and 100%; tr (trace), hemolysis 50% or less; 0, no hemolysis.

The results obtained with Serums 21-30, inclusive, are shown in Table 2. Binding of complement proceeded slowly and was a trifle faster in the refrigerator than in the incubator. Serum 28, after 0.25 hour incubation, gave a faintly positive result by both methods. After 0.5 hour incubation the incubator method gave a weakly positive (+) result and the refrigerator method gave a strongly positive (+++) result. With 4 hour incubation the incubator method gave a +++ positive result and the refrigerator method gave a ++++ positive result. Serum 25 shows the same difference, and none of the other serums gave stronger positive results by the incubator method than by the refrigerator method.

## TEST 3

With serums 31 to 50, inclusive, first incubation was done in the incubator and in the refrigerator for 1 and 4 hours. In these tests larger quantities of human serum were used than in Tests 1 and 2, hence the larger quantities of hemolytic amboceptor. After having added the sensitized blood corpuscles all tubes were placed in the incubator, the water-bath having been discarded. Serums 31 to 40, inclusive, came from nonsyphilitic persons and Serums 41 to 50, inclusive, came from known syphilitics.

TABLE 3  
INCUBATION IN INCUBATOR COMPARED WITH INCUBATION IN REFRIGERATOR

Num- ber	Incubation Method I = Incu- bator R = Refrig- erator	Incuba- tion Time, Hours	Ambo- ceptor per Tube Unit	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
31	I	1	1.5	+	+	±	+	+	±	Negative, Negative, Negative, Negative,	—
	R	1	1.5	+	+	±	+	+	±		—
	I	4	1.5	+	+	tr	+	+	tr		—
	R	4	1.5	+	+	±	+	+	±		—
32	I	1	1.5	+	+	tr	+	+	tr	Negative, Negative, Negative, Negative.	—
	R	1	1.5	+	+	±	+	+	±		—
	I	4	1.5	+	+	0	+	+	0		—
	R	4	1.5	+	+	±	+	+	±		—
33	I	1	1.5	+	+	±	+	+	±	Negative, Negative, Negative, Negative,	—
	R	1	1.5	+	+	±	+	+	±		—
	I	4	1.5	+	+	tr	+	+	tr		—
	R	4	1.5	+	+	±	+	+	±		—
34	I	1	1.5	+	+	±	+	+	±	Negative, Negative, Negative, Negative,	—
	R	1	1.5	+	+	±	+	+	±		—
	I	4	1.5	+	+	tr	+	+	tr		—
	R	4	1.5	+	+	±	+	+	±		—
35	I	1	1.5	+	+	±	+	+	±	Negative, Negative, Negative, Negative,	—
	R	1	1.5	+	+	±	+	+	±		—
	I	4	1.5	+	+	tr	+	+	tr		—
	R	4	1.5	+	+	±	+	+	±		—
36	I	1	1.5	+	+	tr	+	+	tr	Negative, Negative, Negative, Negative,	—
	R	1	1.5	+	+	tr	+	+	tr		—
	I	4	1.5	+	+	tr	+	+	tr		—
	R	4	1.5	+	+	±	+	+	±		—
37	I	1	1.5	+	+	tr	+	+	tr	Negative, Negative, Negative, Negative,	—
	R	1	1.5	+	+	±	+	+	±		—
	I	4	1.5	+	+	tr	+	+	tr		—
	R	4	1.5	+	+	±	+	+	±		—
38	I	1	1.5	+	+	tr	+	+	tr	Negative, Negative, Negative, Negative,	—
	R	1	1.5	+	+	±	+	+	±		—
	I	4	1.5	+	+	tr	+	+	tr		—
	R	4	1.5	+	+	±	+	+	±		—
39	I	1	1.5	+	+	tr	+	+	tr	Negative, Negative, Negative, Negative,	—
	R	1	1.5	+	+	tr	+	+	tr		—
	I	4	1.5	+	+	0	+	+	0		—
	R	4	1.5	+	+	tr	+	+	tr		—
40	I	1	1.5	+	+	tr	+	+	tr	Negative, Negative, Negative, Negative,	—
	R	1	1.5	+	+	tr	+	+	tr		—
	I	4	1.5	+	+	0	+	+	0		—
	R	4	1.5	+	+	tr	+	+	tr		—

Explanation: + means complete hemolysis; ±, hemolysis between 50% and 100%; tr (trace), hemolysis 50% or less; 0, no hemolysis.

TABLE 3—Continued

INCUBATION IN INCUBATOR COMPARED WITH INCUBATION IN REFRIGERATOR

Number	Incubation Method I = Incubator R = Refrigerator	Incubation Time, Hours	Amboceptor per Tube Unit	Readings						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
41	I	1	1.5	+	±	0	+	+	tr	Moderately positive, ++ Strongly positive, ++++ Moderately positive, ++ Strongly positive, ++++++
	R	1	1.5	+	0	0	+	+	tr	
	I	4	1.5	+	±	0	+	+	tr	
	R	4	1.5	0	0	0	+	+	±	
42	I	1	1.5	+	+	0	+	+	0	Faintly positive, ± Weakly positive, + Moderately positive, ++ Strongly positive, +++
	R	1	1.5	+	±	0	+	+	0	
	I	4	1.5	+	tr	0	+	+	0	
	R	4	1.5	+	0	0	+	+	0	
43	I	1	1.5	+	+	tr?	+	+	tr	Faintly positive, ± Weakly positive, + Weakly positive, + Strongly positive, ++++
	R	1	1.5	+	+	tr	+	+	±	
	I	4	1.5	+	+	0	+	+	tr	
	R	4	1.5	+	tr	0	+	+	±	
44	I	1	1.5	+	+	±	+	+	±	Negative, — Strongly positive, ++++++ Negative, — Strongly positive, ++++++
	R	1	1.5	0	0	0	+	+	±	
	I	4	1.5	+	+	tr	+	+	tr	
	R	4	1.5	0	0	0	+	+	±	
45	I	1	1.5	+	+	±	+	+	±	Negative, — Strongly positive, ++++ Negative, — Strongly positive, ++++++
	R	1	1.5	+	tr	0	+	+	tr	
	I	4	1.5	+	+	tr	+	+	tr	
	R	4	1.5	0	0	0	+	+	±	
46	I	1	1.5	+	+	tr	+	+	±	Weakly positive, + Strongly positive, +++ Weakly positive, + Strongly positive, ++++++
	R	1	1.5	+	±	0	+	+	±	
	I	4	1.5	+	+	0	+	+	tr	
	R	4	1.5	0	0	0	+	+	±	
47	I	1	1.5	+	+	±	+	+	±	Negative, — Negative, — Negative, — Strongly positive, +++
	R	1	1.5	+	+	±	+	+	±	
	I	4	1.5	+	+	tr	+	+	tr	
	R	4	1.5	+	±	0	+	+	±	
48	I	1	1.5	+	+	0	+	+	±	Moderately positive, ++ Strongly positive, ++++ Moderately positive, ++ Strongly positive, ++++
	R	1	1.5	+	±	0	+	+	±	
	I	4	1.5	+	+	0	+	+	±	
	R	4	1.5	+	tr	0	+	+	±	
49	I	1	1.5	+	+	0	+	+	±	Moderately positive, ++ Strongly positive, ++++ Strongly positive, +++ Strongly positive, ++++++
	R	1	1.5	+	tr	0	+	+	±	
	I	4	1.5	+	±	0	+	+	±	
	R	4	1.5	±	0	0	+	+	±	
50	I	1	1.5	+	tr	0	+	+	±	Strongly positive, ++++ Strongly positive, ++++++ Strongly positive, ++++++ Strongly positive, ++++++
	R	1	1.5	±	0	0	+	+	±	
	I	4	1.5	+	0	0	+	+	±	
	R	4	1.5	0	0	0	+	+	±	

Explanation: + means complete hemolysis; ±, hemolysis between 50% and 100%; tr (trace), hemolysis 50% or less; 0, no hemolysis.

In Table 3 are recorded the results obtained with Serums 31 to 50, inclusive. Serums 31 to 40, inclusive, gave negative results with all 4 methods. With Serums 41 to 50 the results varied greatly; such results as those obtained with Serums 44 and 45 may be very unusual. All of these serums gave much stronger positive results by the refrigerator method than by the incubator method, and with 4-hour first incubation much stronger positive results were obtained than with 1-hour first incubation.



TABLE 4

FIVE HOUR INCUBATION IN REFRIGERATOR COMPARED WITH TEN HOUR INCUBATION  
IN REFRIGERATOR

Num- ber	Incubation Method R = Refrig- erator	Incuba- tion Time, Hours	Ambo- ceptor per Tube Unit	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
51	R	5	1.5	+	+	±	+	+	±	Negative, Negative,	—
	R	10	1.5	+	+	±	+	+	±		—
52	R	5	1.5	+	+	±	+	+	±	Negative, Negative,	—
	R	10	1.5	+	+	±	+	+	±		—
53	R	5	1.5	+	+	tr	+	+	tr	Negative, Negative,	—
	R	10	1.5	+	+	tr	+	+	tr		—
54	R	5	1.5	+	+	±	+	+	±	Negative, Negative,	—
	R	10	1.5	+	+	±	+	+	±		—
55	R	5	1.5	+	+	±	+	+	±	Negative, Negative,	—
	R	10	1.5	+	+	±	+	+	±		—
56	R	5	1.5	+	+	±	+	+	±	Negative, Negative,	—
	R	10	1.5	+	+	±	+	+	±		—
57	R	5	1.5	+	+	±	+	+	±	Negative, Negative,	—
	R	10	1.5	+	+	±	+	+	±		—
58	R	5	1.5	+	+	tr	+	+	tr	Negative, Negative,	—
	R	10	1.5	+	+	tr	+	+	tr		—
59	R	5	1.5	+	+	±	+	+	±	Negative, Negative,	—
	R	10	1.5	+	+	±	+	+	±		—
60	R	5	1.5	+	+	±	+	+	±	Negative, Negative,	—
	R	10	1.5	+	+	±	+	+	±		—
61	R	5	1.5	+	±	0	+	+	±	Strongly positive, Strongly positive,	+++
	R	10	1.5	+	tr	0	+	+	±		++++
62	R	5	1.5	+	tr	0	+	+	±	Strongly positive, Strongly positive,	++++
	R	10	1.5	+	0	0	+	+	±		+++++
63	R	5	1.5	+	tr	0	+	+	±	Strongly positive, Strongly positive,	++++
	R	10	1.5	+	0	0	+	+	±		+++++
64	R	5	1.5	+	+	tr	+	+	±	Weakly positive, Moderately positive,	+
	R	10	1.5	+	+	0	+	+	±		++
65	R	5	1.5	+	+	0	+	+	±	Moderately positive, Strongly positive,	++
	R	10	1.5	+	tr	0	+	+	±		++++
66	R	5	1.5	+	tr	0	+	+	±	Strongly positive, Strongly positive,	++++
	R	10	1.5	tr	0	0	+	+	±		++++
67	R	5	1.5	+	+	±?	+	+	±	Faintly positive, Weakly positive,	±
	R	10	1.5	+	+	tr	+	+	±		+
68	R	5	1.5	+	+	0	+	+	±	Moderately positive, Strongly positive,	++
	R	10	1.5	+	±	0	+	+	±		+++
69	R	5	1.5	+	+	0	+	+	±	Moderately positive, Strongly positive,	++
	R	10	1.5	+	tr	0	+	+	±		++++
70	R	5	1.5	+	+	tr	+	+	±	Weakly positive, Moderately positive,	+
	R	10	1.5	+	+	0	+	+	±		++

Explanation: + means complete hemolysis; ±, hemolysis between 50% and 100%; tr (trace), hemolysis 50% or less; 0, no hemolysis.

## TEST 4

With Serums 51 to 70, inclusive, first incubation was done in the refrigerator at a temperature of 10 C. for 5 hours and 10 hours. After having added the sensitized blood corpuscles the tubes were placed in the incubator for 2 hours and the results were read from 3-5 hours after the blood corpuscles had been added.

Table 4 shows the results obtained with Serums 51 to 70, inclusive. Serums 51 to 60, inclusive, gave negative results with both methods. Serums 61 to 70, inclusive, gave positive results, and the results were stronger positive after 10-hour first incubation than they were after 5-hour first incubation. The most marked differences were obtained with Serums 64, 65, 66, 69, and 70. All of these serums gave twice as strongly positive results with 10-hour incubation as they did with 5-hour incubation.

## TEST 5

With Serums 71 to 80, inclusive, first incubation was done in the refrigerator at a temperature of 10 C. for 5 hours and at room temperature of 21 C. for 5 hours. After having added the sensitized blood corpuscles all tubes were put in the incubator at a temperature of 37 C. for 2 hours and the results were read about 3 hours after the corpuscles had been added.

TABLE 5  
FIVE HOUR INCUBATION AT 10 C. COMPARED WITH FIVE HOUR INCUBATION AT 21 C.

Number	Incubation Temperature, C.	Incubation Time, Hours	Amboceptor per Tube Unit	Readings						Results	
				Antigen Tubes			Control Tubes				
				1	2	3	1'	2'	3'		
71	10	5	1.5	+	+	tr	+	+	±	Weakly positive, Negative	+
	21	5	1.5	+	+	±	+	+	±		—
72	10	5	1.5	+	0	0	+	+	±	Strongly positive, Weakly positive,	+++++
	21	5	1.5	+	+	tr	+	+	±		+
73	10	5	1.5	+	0	0	+	+	±	Strongly positive, Strongly positive,	+++++
	21	5	1.5	+	±	0	+	+	±		+++
74	10	5	1.5	+	±	0	+	+	±	Strongly positive, Weakly positive,	+++
	21	5	1.5	+	+	tr	+	+	±		+
75	10	5	1.5	+	+	0	+	+	±	Moderately positive, Faintly positive,	++
	21	5	1.5	+	+	±?	+	+	±		±
76	10	5	1.5	+	±	0	+	+	±	Strongly positive, Weakly positive,	+++
	21	5	1.5	+	+	tr	+	+	±		+
77	10	5	1.5	±	0	0	+	+	±	Strongly positive, Strongly positive,	+++++
	21	5	1.5	+	tr	0	+	+	±		++++
78	10	5	1.5	+	0	0	+	+	±	Strongly positive, Strongly positive,	+++++
	21	5	1.5	+	±	0	+	+	±		+++
79	10	5	1.5	+	0	0	+	+	±	Strongly positive, Strongly positive,	+++++
	21	5	1.5	+	±	0	+	+	±		+++
80	10	5	1.5	0	0	0	+	+	±	Strongly positive, Strongly positive,,	+++++
	21	5	1.5	+	±	0	+	+	±		+++++

Explanation: + means complete hemolysis; ±, hemolysis between 50% and 100%; tr (trace), hemolysis 50% or less; 0, no hemolysis.

The results obtained with Serums 71 to 80, inclusive, at temperatures of 10 C. and 21 C. are shown in Table 5. With all of these serums complement binding was better at the lower than at the higher temperature.

## CONCLUSIONS

Complement binding is better in the incubator at a temperature of 37 C. than in the open water-bath at the same temperature.

Complement binding takes place gradually, many hours being required for its completion.

Complement binding is much better at a temperature of 10 C. than at a temperature of 37 C. or of 21 C.

Perhaps the optimum temperature for complement binding and the length of time necessary for its completion in the Wassermann reaction have not yet been determined.